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Naturally *agr*-negative livestock-associated MRSA exhibits high adhesive capacity to human and porcine cells

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Verstärktes Adhäsionsverhalten einer natürlichen *agr*-negativen MRSA-Mutante vom Schwein an humane und porzine Zellen

Methicillin-resistente *Staphylococcus aureus* (MRSA) des CC398 gehören zu häufig vorkommenden Kommensalen bei Nutztieren, können Infektionen bei Menschen verursachen und weisen eine hohe Mutationsrate auf. Neulich wurde im Rahmen einer Studie bei finnischen Schlachtschweinen ein MRSA-Stamm des neuen *spa* Typs CC398/t2741 entdeckt, dem die Gene *agr* (Regulator für Virulenzfaktoren) und *fnbB* (Adhäsionsfaktor) fehlen. Ziel der vorliegenden Arbeit war es, den Phänotypen dieses natürlich vorkommenden *agr/fnbB*-negativen MRSA zu charakterisieren und den genomischen Hintergrund zu analysieren. Dazu wurden Wachstumskurven, Hämolysemuster, Adhäsionsverhalten auf Zelllinien humaner Keratinozyten und porziner Nasenmukosazellen sowie die genomischen Sequenzdaten untersucht. Der *agr*-negative Stamm zeigte eine signifikant bessere Adhäsion an humane und porzine Zellen als die beiden *agr*-positiven Kontrollstämme (CC398/t274 bzw. CC398/t034). Die beiden Kontrollstämme, nicht aber die Mutante, hatten einen zytotoxischen Effekt auf die porzinen Zellen. Die ausgeprägte Adhäsionskapazität des *agr*-negativen porzinen MRSA-Feldstammes zusammen mit der fehlenden Zytotoxizität könnte eine persistente Kolonisation bei Schweinen begünstigen. Dieser Stamm sowie eine humane Δ*agr* Knockout-Mutante zeigten unabhängig von der Zelllinie eine ähnlich starke Adhäsionskapazität. Diese Resultate weisen auf ein erhöhtes Potential dieses *agr*-negativen porzinen MRSA-Feldstammes bezüglich Übertragung auf den Menschen hin.

Stichwörter: *Staphylococcus aureus;* MRSA CC398; Schwein; Adhäsion; *agr*

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Naturally *agr*-negative livestock-associated MRSA exhibits high adhesive capacity to human and porcine cells

Methicillin-resistant *Staphylococcus aureus* (MRSA) of CC398 have emerged as important colonizers of livestock, can cause human infections and evolve rapidly. A recent study reporting a new dominant *spa* type among MRSA from Finish fattening pigs (CC398/t2741) identified a strain lacking the global virulence regulator gene *agr* and the adhesion gene *fnbB*. The aim of this study was to characterize the phenotype of this *agr/fnbB*-negative livestock-associated MRSA and to provide data on its genetic background. Thus, growth curves, hemolysis patterns, adhesion assays on human keratinocyte and porcine nasal mucosa cell lines and whole genome sequencing were performed. The *agr*-negative strain adhered significantly better to human and porcine host cells than two *agr*-positive control pig strains (1. CC398/t274; same herd / 2. CC398/t034; another herd). For the *agr*-positive porcine MRSA strains, cytotoxic effects on porcine mucosal cells were observed. The strong adhesive capacity of the naturally *agr*-negative MRSA in combination with diminished cytotoxic effects is likely favorable for inducing persistent colonization in pigs. Independent of the host cell type, similar adhesive capacities of the naturally *agr*-negative porcine MRSA and the human MRSA (an isogenic Δ*agr* knockout mutant strain) were shown. These results indicate that loss of *agr* in the livestock-associated MRSA strain investigated in this study may have increased its potential to be transmitted to and amongst humans.

Keywords: *Staphylococcus aureus;* MRSA; livestock; adhesion; *agr*

Naturally *agr*-negative livestock-associated MRSA exhibits high adhesive capacity to human and porcine cells

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1. Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) of CC398 have emerged as colonizers of livestock, can cause human infections and evolve rapidly. A study reporting a new *spa* type among MRSA from Finish fattening pigs (CC398/t2741) identified a strain lacking the global virulence regulator gene *agr* and the adhesion gene *fnbB*. The aim of this study was to characterize the phenotype of this *agr/fnbB*-negative livestock-associated MRSA and to provide data on its genetic background. Thus, growth curves, hemolysis patterns, adhesion assays on human keratinocyte and porcine nasal mucosa cell lines and whole genome sequencing were performed. The *agr*-negative strain adhered significantly better to human and porcine host cells than two *agr*-positive control pig strains. For the *agr*-positive porcine MRSA strains, cytotoxic effects on porcine mucosal cells were observed. The strong adhesive capacity of the naturally *agr*-negative MRSA in combination with diminished cytotoxic effects is likely favorable for inducing persistent colonization in pigs. Independent of the host cell type, similar adhesive capacities of the *agr*-negative porcine MRSA and the human MRSA (an isogenic Δ*agr* knockout mutant) were shown. These results indicate that loss of *agr* in this livestock-associated MRSA strain may have increased its potential to be transmitted to and amongst humans.

Keywords: *Staphylococcus aureus;* MRSA; livestock; adhesion; *agr*

2. Introduction

Livestock-associated MRSA (LA-MRSA) strains have emerged worldwide. The strains typically belong to CC398 and are detected in particularly high rates among pigs [1-4]. LA-MRSA CC398 are thought to have originated from human methicillin-susceptible *Staphylococcus aureus* and acquired methicillin resistance during the host jump, at the same time losing genes important for colonization and infection in humans [5]. Even though LA-MRSA of CC398 lack important virulence factors, they have been suggested to exhibit an enhanced ability to acquire virulence factors through mobile genetic elements [6].

Transmission of CC398 from animals to humans has been described and occupational exposure to pigs was shown to increase nasal carriage rates (pig farmers: 20-86%; veterinarians: 4.6-45%) compared to the general population (0.8-3%) [2, 4, 7-14]. In addition, in recent years, there have been an increasing number of reports of MRSA CC398 strains causing invasive infections in humans without contact to livestock [15-18]. Therefore, the transmissibility and the virulence potential of LA-MRSA of CC398 need to be closely monitored.

Heikinheimo et al. [19] recently identified a new dominant *spa* type among LA-MRSA strains colonizing Finnish fattening pigs (CC398/t2741). Interestingly, for one CC398/t2741 strain isolated in this study, no genes within the accessory gene regulator locus (*agr*) or the fibronectin-binding protein B (*fnbB*) were detected by DNA microarray. The quorum sensing *agr* system is a major virulence gene regulator. Activation of *agr* has been shown to inhibit expression of certain cell-wall associated proteins and to increase exoprotein expression during the post-exponential phase of growth [20, 21]. While *agr* plays a major role in acute

infections, *agr*-defective mutants can frequently be isolated from bacteraemic patients and were linked to persistent infections [22] and increased mortality [23]. The *fnbB* gene encodes the fibronectin binding protein B (FnBPB), which belongs to the group of microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). It facilitates adhesion of *S. aureus* to the components of the host cells and has a role in invasion [24]. Combined FnBPA and FnBPB function has been shown to be essential to induce severe infection [25].

The aim of this study was to characterize the naturally *agr/fnbB*-negative CC398/t2741 LA-MRSA strain detected in Finland with regard to growth, hemolysis, and adhesive capacity, and to describe the genomic background of the strain.

3. Materials and methods

3.1 Bacterial strains

An overview of the strains used in this study is presented in Table 1. The naturally *agr/fnbB*-negative LA-MRSA strain investigated in this study (Fin47_H17) was isolated from the carcass of a fattening pig in Finland [19]. Two porcine LA-MRSA strains isolated in the same study were used as controls: Fin46_H17, an *agr*-positive, *fnbB*-negative isolate from the same herd (herd 17), and Fin48_H18, an *agr/fnbB*-positive isolate from another herd (herd 18). As additional controls, a MRSA strain that had caused recurring skin infections in humans (MN10) [26] and its isogenic *agr* knockout mutant (MN10_ Δ*agr*) were used. MN10_Δ*agr* was obtained by transduction of the *agr* knockout from RN6911 to MN10 using phage 80α and protocols previously described [27]. Correct deletion of *agr* in putative mutants grown on tetracycline selective plates was confirmed by PCR.

Table 1: Strains used in this study. Presence/absence of *agr* and *fnbB* is indicated as previously determined by DNA microarray [19].

Strain ID	Characteristics	Country	Year	Source [reference]
Fin47_H17	CC398/t2741, *agr*$^-$, *fnbB*$^-$	FI	2015	Pig carcass, herd 17 [19]
Fin46_H17	CC398/t2741, *agr*$^+$, *fnbB*$^-$	FI	2015	Pig nares, herd 17 [19]
Fin48_H18	CC398/t034, *agr*$^+$, *fnbB*$^+$	FI	2015	Pig nares, herd 18 [19]
MN10	CC5/t002, *agr*$^+$, *fnbB*$^+$	CH	2013	Human skin infection [26]
MN10_Δ*agr*	CC5/t002, NM10 *agr::tetM*, *agr*$^-$, *fnbB*$^+$	CH	2013	This study
RN27	80alpha lysogen	-	-	Brigitte Berger-Bächi [28]
RN6911	*agr* operon deleted (*agr::tetM*)	-	-	Brigitte Berger-Bächi [28]

3.2 Growth parameters

Single colonies were transferred from 5% sheep blood agar to 50 mL of Luria Bertani (LB) broth (Becton Dickinson, Allschwil, Switzerland) and grown for 24 h at 37°C (225 rpm). Growth of all strains in LB was determined by viable cell counts after 3 h, 6 h, 9 h, and 24 h using 10-fold dilution series and plate count agar (Sigma-Aldrich, Stockholm, Sweden), with incubation at 37°C for 18-24 h. Growth parameters such as exponential phase growth rate and maximum cell density were determined using DMFit 3.0 [29].

3.3 Hemolysis

Screening for hemolytic activity was performed as previously described [30]. Briefly, alpha- and delta-hemolytic activity was determined by perpendicular streaking to the beta-hemolysin producing *S. aureus* reference strain RN4220 on 5% sheep blood agar and incubation over night at 37°C. In this assay, beta hemolysis results in a turbid zone. Alpha hemolytic activity of the test strain is inhibited by beta hemolysis of RN4220 where the strains intersect. Delta hemolysis of the test strain is synergistic with beta hemolysin of RN4220, resulting in an amplified zone of clearing where the strains intersect [31]. Gamma hemolytic activity cannot be detected in this assay, as it is inhibited by agar [32]. Subsequent cold shock (exposure to 4°C for 12h) was used to determine beta-hemolysis activity.

3.4 Adhesion assay

Two different cell lines were used for adhesion assays: human cell line HaCaT (Human adult low Calcium high Temperature keratinocytes, CLS Cell Lines Service GmbH,

Eppelheim, Germany; [33]) and porcine cell line PT-K75 (porcine nasal turbinate/mucosa, CRL-2528, ATCC, Manassas, USA). The medium for the adhesion assay consisted of DMEM supplemented with 4.5 g/L glucose, 584 mg/L L-glutamine and 10% FCS (CLS Cell Lines Service GmbH) for the HaCaT cells and DMEM supplemented with 4.5 g/L glucose, 580mg/L L-glutamine, 110mg/L sodium pyruvate (GIBCO, Thermo Fisher Scientific, Invitrogen, Carlsbad, CA, USA) and 10% FCS (BioConcept, Allschwil, Switzerland) for the PT-K75 cells.

After at least one passage in a 75cm^2 canted neck tissue culture flask with vented cap (Corning, Sigma-Aldrich), HaCaT-cells were seeded at a concentration of 2×10^5 cells/well and PT-K75-cells at a concentration of 1.25×10^5 cells/well in a 24-well flat-bottom cell culture plate with low-evaporation lid (Techno Plastic Products AG, TPP, Trasadingen, Switzerland). The culture plates were incubated for 24 hours at 37°C and 5% CO_2 to reach a confluency of at least 80%.

Bacterial strains were prepared by growth on 5% sheep blood agar overnight at 37°C. Overnight cultures were prepared by inoculation of 5 mL LB broth with a single colony and incubation at 37°C (225 rpm shaking) for 15 hours. Cultures were subsequently adjusted to $OD_{590} = 0.40$ (~ 10^8 CFU/ml) for each strain. The bacteria were used at a MOI of 10 to infect both cell lines (HaCaT and PT-K75) in 24-well plates. Uninfected cells were used as negative controls. After 15 min of incubation at 37°C, cells were imaged, before monolayers were washed eight times with Dulbecco's phosphate buffered saline (DPBS, GIBCO) and cells were harvested after five minutes of incubation with Trypsin-EDTA (0.25%, GIBCO) by scraping with a cell scraper (TPP). Bacterial cell counts were determined by 10-fold dilution series and plate count agar. Three independent biological experiments were performed, each using three replicates per strain. For imaging purposes, the Nikon Eclipse Ti-U inverted

microscope was used at 200-fold magnification with software NIS-Elements AR Analysis 4.3 (Nikon AG, Egg, Switzerland).

3.5 Statistical analysis

All statistical analyses with the exception of growth parameter analyses were performed using R, 3.2. Results were compared using a gaussian linear model of the form count~strain+host.cellline. Growth parameters were compared between strains using SPSS Statistics 22 (SPSS Inc.Chicago, Illinois). Growth parameters such as exponential phase growth rate and maximum cell density were determined using DMFit 3.0 [29] and compared using one-way ANOVA. Differences were considered statistically significant if $p < 0.05$.

3.6 Whole genome sequencing

DNA was extracted from overnight cultures grown on 5% sheep blood agar plates using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The porcine LA-MRSA strains Fin47_H17, Fin46_H17, and Fin48_H18 were sequenced on the Illumina Miseq platform with 250bp paired end reads following NEBNextUltra library creation. Raw sequencing reads were adaptor and quality trimmed and filtered using Trimmomatic version 0.32 [34]. Mapping against the CC398 reference strain S0385 (EMBL Accession AM990992) [6] using BWA [35] indicated chromosome coverage of each genome to be: Fin47_H17 = 132.8x ± 76.2, Fin46_H17 = 140.6 x ±71.2, Fin48_H18 = 115.1x ± 53.7. *De novo* assembly used SPAdes v 3.1.0 in multi-cell mode [36]. Scaffolds were rearranged in ACT [37] against S0385 using files generated in

WebACT (http://www.webact.org/). Resulting assemblies comprise 28, 25 and 27 scaffolds respectively, with scaffolds under 1kb, which did not match the reference, excluded. Target coding sequences (CDSs) involved in adhesion were identified and analysed in all genomes using ACT and, where necessary, single CDSs were aligned using ClustalW. The phylogeny was generated in http://wgsastaging.pathogensurveillance.net/ using available assembled CC398 genomes for context [6, 38-42]. Antibiotic resistance phenotypes were also predicted using this online tool.

All read data and assemblies have been deposited with ENA under project number (PRJEB14187).

4. Results

4.1 Growth parameters

The growth of the five strains in LB broth was almost equal with regard to exponential phase growth rate or maximum cell density (Fig. 1). However, the naturally *agr*-negative LA-MRSA Fin47_H17 exhibited the lowest exponential phase growth rate (μ_{max} = 0.50) compared to all other strains (μ_{max} = 0.51-0.57). Fin47_H17 also reached the lowest maximum cell density (8.97 log CfU/mL) compared to all other strains tested (9.70-9.91 log CfU/mL).

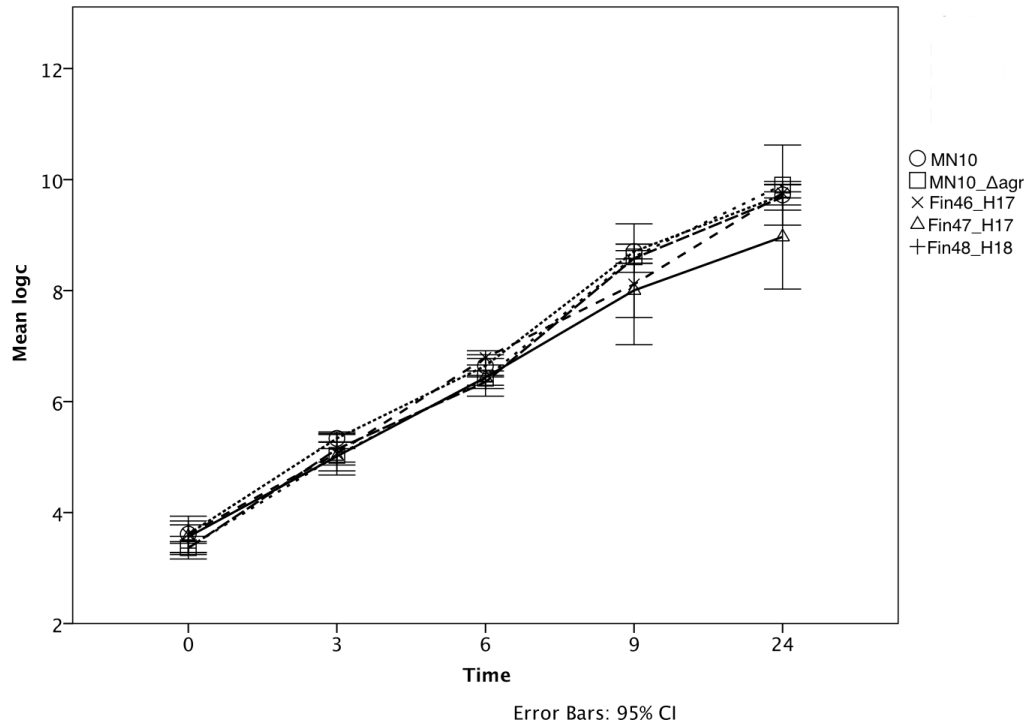

Fig. 1: Growth of all tested strains in LB over 24h. The tested strains exhibited no statistically significant differences in exponential phase growth rate or maximum cell density reached.

4.2 Haemolysis pattern

Hemolysis patterns after incubation at 37°C overnight and after subsequent cold exposure are shown in Fig. 2. No hemolysis was visible for both the naturally *agr*-negative Fin47_H17 strain and *agr* knockout mutant MN10_ Δ*agr*. Parental strain MN10 exhibited alpha and delta hemolysis. Fin46_H17 exhibited alpha and beta hemolysis, and Fin48_H18 exhibited beta and delta hemolysis.

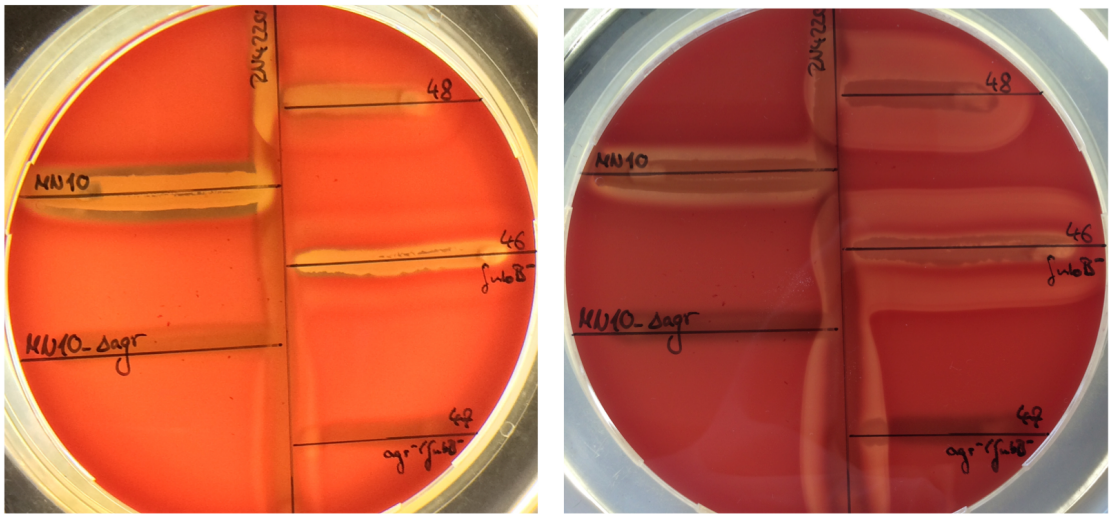

Fig. 2: Hemolysis. The *agr*-negative strains exhibited no hemolysis (Fin47_H17 and MN_ Δ*agr*). MN10 exhibited alpha and delta hemolysis, Fin46_H17 exhibited alpha and beta hemolysis, and Fin48_H18 exhibited beta and delta hemolysis.

4.3 Adhesion properties

While no morphological changes were observed in HaCaT cells after bacterial infection, infection of PT-K75 cells with the *agr*-positive porcine LA-MRSA strains (Fin46_H17 and Fin48_H18) resulted in a cytotoxic effect including shrinking, rounding and detachment of the cells (Fig. 3). To minimize cytotoxicity, a trial experiment was performed

on PT-K75 cells with an MOI of 1: cytotoxic effects were still observed (data not shown). In contrast, neither the naturally *agr*-negative porcine LA-MRSA strain Fin47_H17 nor the human-associated MRSA strain MN10 and its isogenic *agr* knockout mutant induced any morphological changes in the PT-K75 cells (Fig. 3).

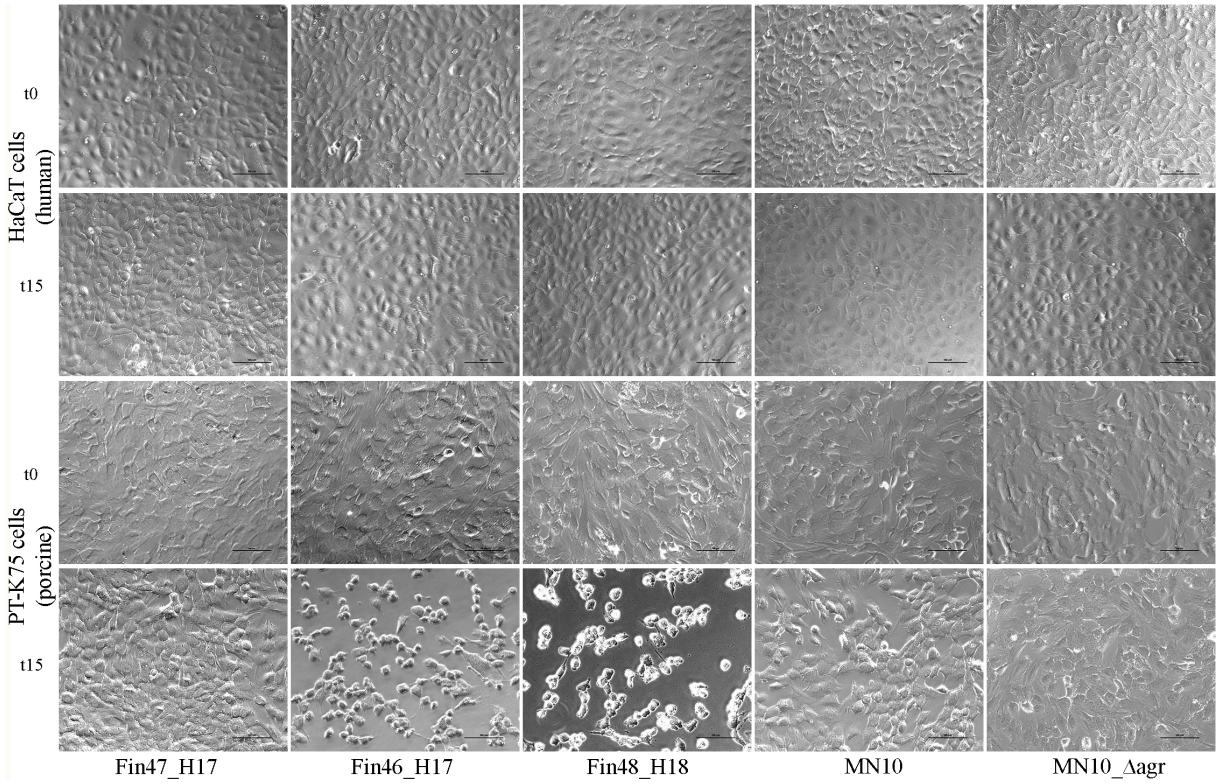

Fig. 3: Cytotoxic effects on porcine cells. Representative images of the two cell lines before (T_0) and shortly after ($T_{15\ min}$) infection. Bacterial strains used were the porcine LA-MRSA strains Fin47_H17, Fin46_H17, and Fin48_H18, as well as the MRSA strain linked to human infections (MN10) and its isogenic knockout mutant MN10_Δ*agr*. Cytotoxic effects of the *agr*-positive porcine LA-MRSA strains Fin46_H17 and Fin48_H18 on PT-K75 cells can be identified at $T_{15\ min}$.

Counts of adherent bacteria revealed that the *agr*-negative strains Fin47_H17 and MN10_Δ*agr* displayed increased adhesive capacity in both human HaCaT and porcine PT-

K75 cells (Fig. 4) compared to the *agr*-positive LA-MRSA and the human-associated parental MRSA strain MN10, respectively. Mean colony counts for Fin47_H17 were 3,300 for HaCaT and 28,644 for PT-K75 cells per culture well. These results show significantly higher adhesive capacity ($p = 0.000$) of this strain compared to the *agr*-positive strains Fin46_H17 (780 to HaCaT), Fin48_H18 (523 to HaCaT) or MN10 (1313 to HaCaT, $p = 0.000$; 6477 to PT-K75, $p = 0.001$). The mean colony counts for the MN10_Δ*agr* mutant strain (2711 to HaCaT and 35711 to PT-K75) were significantly higher than those of MN10 (1313 to HaCaT, $p = 0.003$; 6477 to PT-K75, $p = 0.000$). Thus, the naturally *agr*-negative LA-MRSA and MN10_Δ*agr* exhibited significantly higher adhesive capacity than the *agr*-positive LA-MRSA strains and parental strain MN10, respectively. Overall, the tested porcine and human MRSA strains adhered significantly better to the porcine than to the human cells ($p = 0.000$).

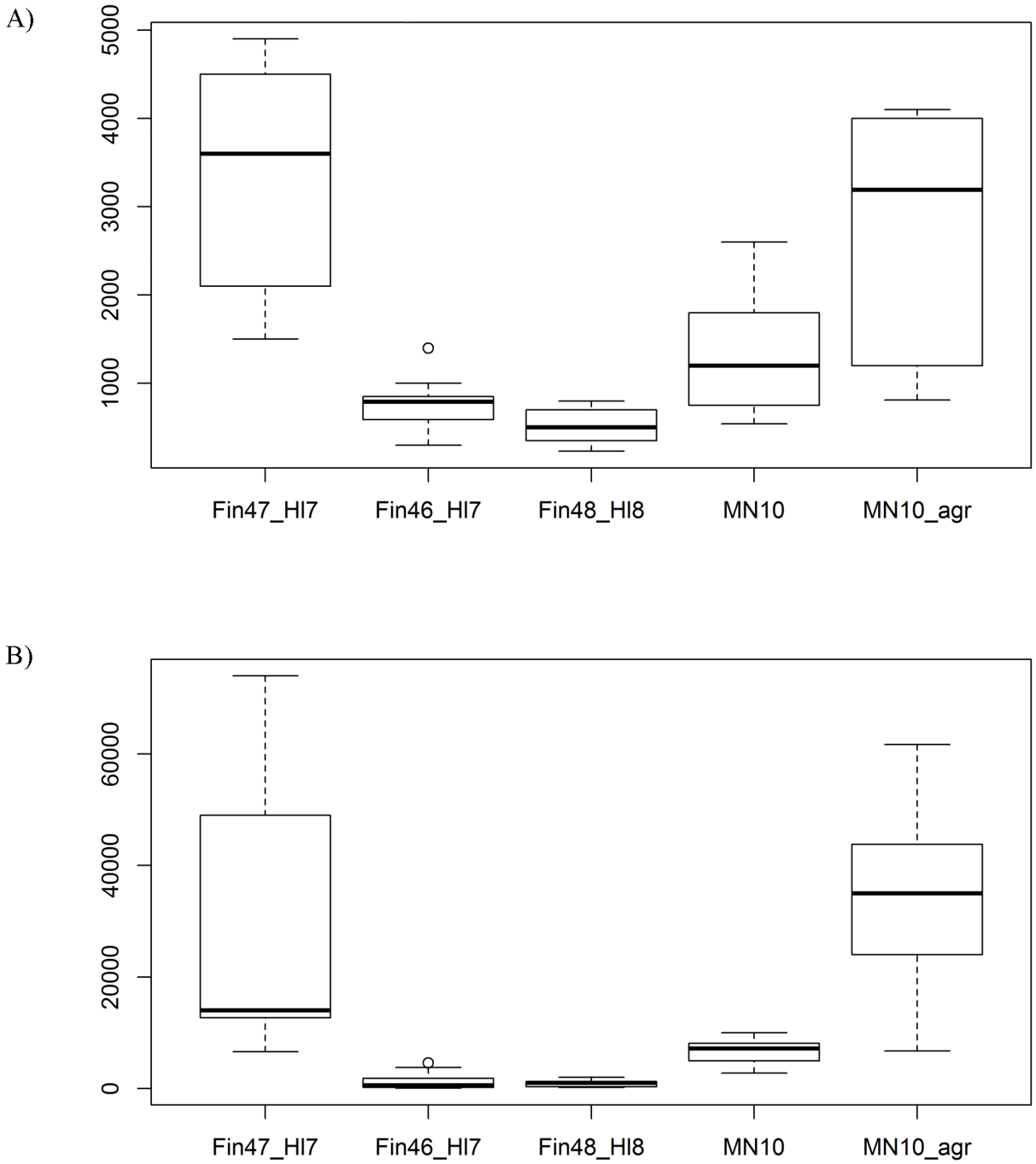

Fig. 4: Adhesion to A) human cells and B) porcine cells: colony counts for different strains. The plots indicate the median (horizontal line), the range from the first to the third quartile (25–75%; box), and the extreme values (whiskers) of the colony counts for each strain after adhesion to A) human cells (HaCaT) and B) porcine cells (PT-K75). The data

resulted from three independent biological experiments with three replicates for each strain (n=9).

4.4 Genomic analysis

Whole genome sequencing and phylogenetic analysis of strains within CC398 confirmed that Fin47_H17 is closely related to Fin46_H17, with Fin48_H18 more distantly related (S1 Fig).

In the naturally *agr*-negative LA-MRSA Fin47_H17, a deletion of 3,789 bp covering *hld*, *agrB* and *agrC* (Fig. 5A) was identified. In both this strain and the closely related strain Fin46_H17, a further deletion completely removing *fnbB* was detected that leaves the adjacent *fnbA* intact (Fig. 5B). However, as *fnbB* in both Fin48_H18 and reference strain S0385 is truncated after 349 amino acids with a single nucleotide mutation causing a premature stop codon, the functional relevance of this is unclear.

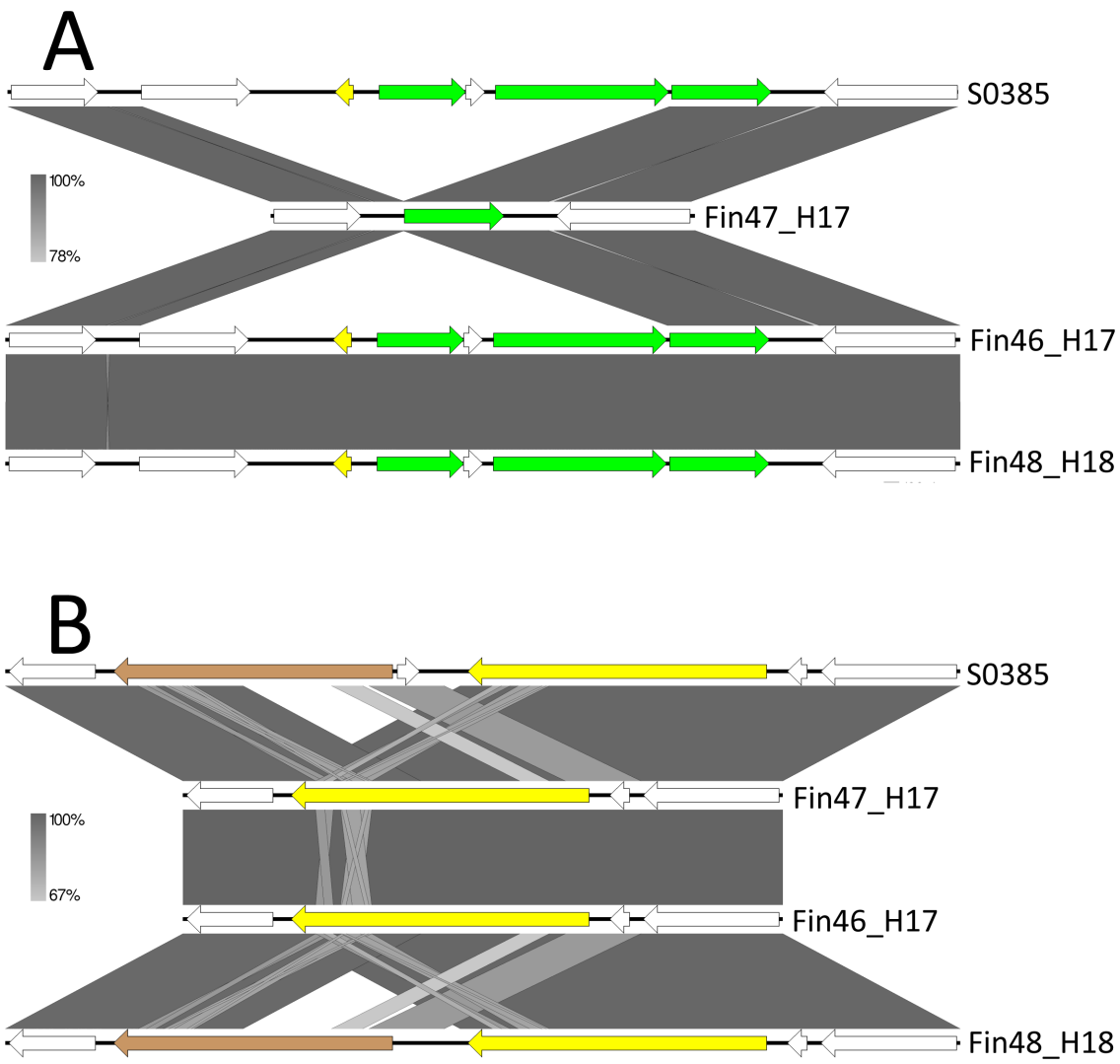

Fig. 5: Comparison of genome loci around *agr* and *fnbB*. Each line refers to a genomic section in the strain indicated. Arrows indicate CDSs. Grey bars show homology between loci according to the scale. **A.** *agr* locus showing the extent of the deletion in Fin47_H18, covering *SAPIG2071-2075*. *hld* is shown in yellow and the *agr* locus (*agrBCA*) in green. Only *agrA* remains in Fin47_H17. **B.** *fnbB* locus showing the deletion of *fnbB* (*SAPIG2551*) in both Fin47_H17 and Fin46_H17. *fnbB* in both S0385 and Fin48_H18 is truncated by a stop codon and is shown in brown for a pseudogene. *fnbA* (yellow) displays some homology to *fnbB*. Figure was drawn in Easyfig [43].

Genes associated with adhesion were investigated within the genomes (S2 Table). Such genes often have varying repeat lengths, and this was identified in these strains in the genes: *spa*, *sdrC* and the hypothetical protein encoding CDS *SAPIG1791*; with the further genes *coa*, *sdrD*, *clfA*, *clfB, ebhA* and *cna* almost identical between the three porcine LA-MRSA strains, but variable in comparison to homologues in S0385. The *vwb* gene, encoding a secreted von Willebrand factor-binding protein, was found to be absent at the equivalent chromosomal locus in Fin48_H18, with a 13.7 kb deletion covering this region. All other genes investigated were found to be identical or almost identical to those in S0385 (*eno*, *isdB*, *isdA*, *ecb*, *fib*, *ebpS*, *map*, *sdrH*, *agrA*, *isaB*).

The strains carry variable collections of plasmids and phage (S2 Table) and the predicted antibiotic resistance profiles (Fin47_H17 and Fin46_H17: PEN^R, TET^R, ERY^R, MET^R; Fin 48_H18: PEN^R, TET^R, MET^R, TRI^R) largely correspond to those phenotypically determined previously, with the exception of clindamycin [19].

5. Discussion

Heikinheimo et al. [19] investigated LA-MRSA isolated from Finnish fattening pigs at slaughter. They found that CC398/t2741 strains were predominant among the isolated LA-MRSA. Interestingly, one of the isolated strains (Fin47_H17) did not harbor genes from the *agr* locus. In this study, we determined the genomic basis of this loss of *agr* and its effect on growth, hemolytic activity, and adhesive capacity to human and porcine cell lines.

Loss of *agr* did not result in significantly altered growth parameters, but had a strong impact on hemolytic activity consistent with previous studies suggesting that expression of alpha and delta hemolysins is strongly induced by *agr* [30, 31]. Hemolysins are widely recognized as important virulence factors in *S. aureus* infections. However, selective survival of *agr*-defective non-hemolytic *S. aureus* in wound and abscess models has been demonstrated [44].

In this study, both *agr*-negative strains tested exhibited increased adhesive capacity to both human and porcine cells. These results are consistent with previous findings suggesting *agr* to inhibit the expression of adhesion proteins [45-48]. Loss of *agr* was shown to significantly increase adhesive capacity to human endothelial [49] and mesothelial cells [50]. In a bovine mammary epithelial cell line (MAC-T), two- to threefold higher numbers of internalized viable bacteria of an *agr* mutant were recovered compared to the wild type [51].

LA-MRSA CC398 was reported to cause more cell damage within epithelial cells than community-associated and hospital-acquired MRSA [52]. In this study, pronounced cytotoxic effects of the *agr*-positive porcine LA-MRSA strains on porcine cells were identified. In contrast, the closely related *agr*-negative porcine LA-MRSA Fin47_H17 did not induce cytotoxic effects. This may be due to reduced expression levels of *agr*-dependent cytotoxic proteins such as alpha hemolysin [53]. It is also consistent with a previous study reporting

cytotoxic effects in MAC-T cells upon infection with RN6390, but no cytotoxic effects in *agr* mutant strain RN6911 [51]. The finding that porcine *agr*-positive LA-MRSA only led to cytotoxicity in the porcine cell line could be due to host-specific factors influencing susceptibility to alpha hemolysin [54].

Whole genome sequencing revealed that the *fnbB* gene in Fin48_H18 is truncated, potentially impairing functionality of FnBPB, whereas it is fully deleted from the genomes of strains Fin47_17 and Fin46_17. We observed no significant differences concerning adherence to human or porcine cells of these strains. For strain Newman, it has been suggested that truncation of *fnbB* was transferred to *fnbA,* resulting in complete secretion FnBPs to the culture medium and loss of cell wall anchor function [55]. Consistent with these findings, reduced adhesive capacity of a *fnbAB* mutant to HaCaT cells has been described [56].

We observed no significant differences in adhesive capacity between porcine and human MRSA apart from the described influence of *agr*. However, irrespective of strain origin (LA-MRSA or human MRSA), adherence to porcine cells significantly exceeded adherence to human cells. LA-MRSA CC398 do not only colonize livestock, but can also be transmitted to humans with direct livestock exposure or even between humans. Although decreased adherence of LA-MRSA compared to community/hospital-associated MRSA to human endothelial and epithelial cells was observed [52], LA-MRSA strains may rapidly adapt to the human host. Our results suggest that LA-MRSA are capable of adherence to both porcine and human cells with the same efficiency as a community-acquired MRSA control strain linked to recurring infections within a family [26].

We were able to show that the recently reported naturally *agr*-negative and *fnbB*-negative LA-MRSA strain Fin47_H17 exhibits high adhesive capacity to porcine and human cells. Our

results suggest that loss of *agr* leads to increased adhesive capacity and that porcine LA-MRSA can exhibit pronounced *agr*-dependent cytotoxic effects on porcine cells. Our findings provide further evidence for the importance of LA-MRSA as an emerging public health concern.

6. Conflict of interest statement

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publication of this article.

7. Acknowledgments

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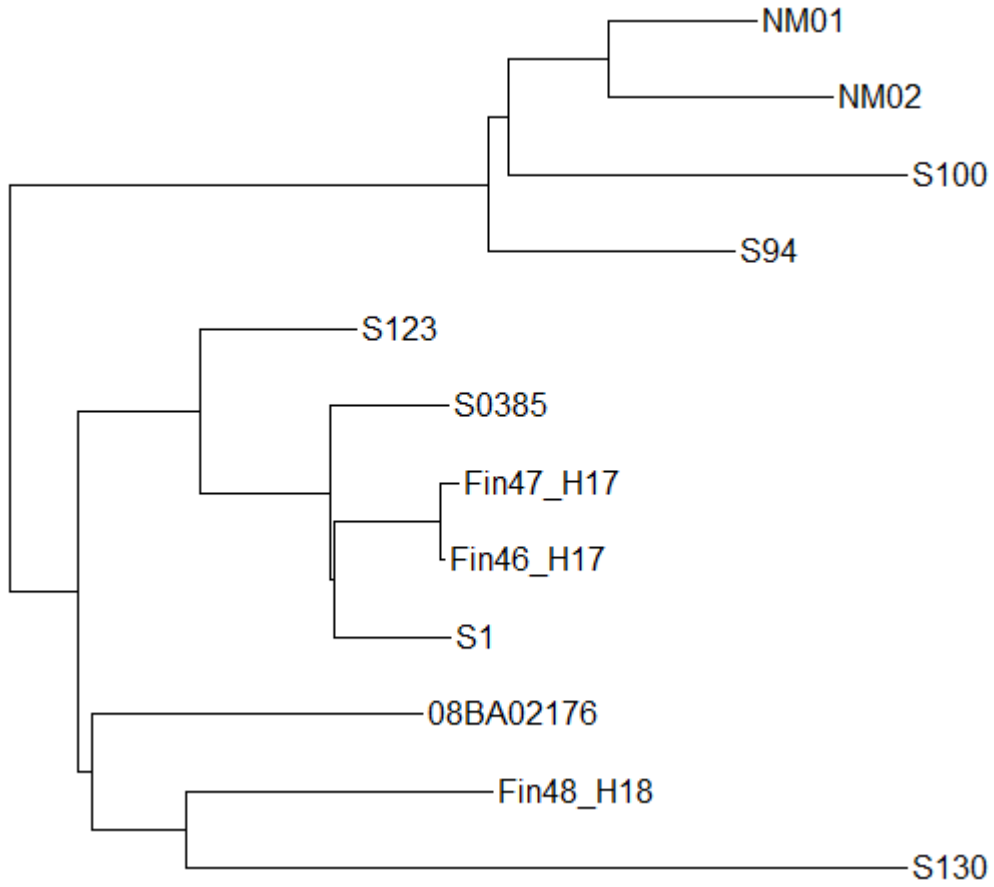
independent of fibronectin-binding proteins. J Invest Dermatol. 2013;133(8):2004-12. doi: 0.1038/jid.2013.87.

9. Supplementary material

S1 Fig. Phylogenetic tree. Phylogenetic tree of whole genome sequenced isolates in the context of other sequenced CC398 strains. Scale bar shows the number of substitutions within the expected core genome (1,799,838 bp).

S2 Table. Variable elements. Table providing an overview of variable elements within the genomes of porcine LA-MRSA strains Fin47_H17, Fin46_17, and Fin48_H18 compared to reference strain S0385.


NM01
NM02
S100
S94
S123
S0385
Fin47_H17
Fin46_H17
S1
08BA02176
Fin48_H18
S130

S1 Fig

S2 Table: Genomic analysis of adhesion associated genes. S0385 data from Uhlemann 2012, with additions. Abbreviations: V/S = variable/same (compared to strain indicated in superscript), A = absent at this location, I = insertion, X = aa substitution, ψ = pseudogenes, wt = wild type, aa =amino acid, Δ = deletion, SNP = single nucleotide polymorphism.

Gene	**S0385 locus tag[1] SAPIG**	**S085**	**NM01**	**Fin47_H17**	**Fin46_H17**	**Fin48_H18**	**Comment**
spa	0122	8 aa Δ at aa390	2 X at aa390	wt	wt	I (8 aa at aa390)	Variable repeat length
coa	0240	81 bp Δ	wt	wt	wt	wt	Variable repeat length
vwb	0483	wt	A	V^{S0385}	V^{S0385}	A (at this location)	Variable sequence/presence
sdrC	0636	174 bp Δ	wt	1 SNP	S^{Fin47_H17}	I (24aa at aa737)	Variable repeat length
sdrD	0637	42 bp Δ and 4 X	Δ 54 bp	wt	wt	wt	Variable repeat length

							and SNPs
eno	0855			S^{S0385}	S^{S0385}	1 SNP, non synonymous	
clfA	0866-7	ψ truncated, 2 frameshifts	wt	V^{S0385}	S^{Fin47_H17}	V^{S0385}	Variable repeat length
isdB	1125	SNP, 9 bp Δ	wt	S^{S0385}	S^{S0385}	I^{S0385}	
isdA	1126			S^{S0385}	S^{S0385}	1 SNP, synonymous	
ecb	1150	wt	wt	S^{S0385}	S^{S0385}	S^{S0385}	
fib	1154	wt	wt	S^{S0385}	S^{S0385}	S^{S0385}	
ebhA	1434	53 aa Δ at aa9851 and 1 X	wt	1 aa X	1 X	2 X	Variable repeat length
ebpS	1480	wt	wt	S^{S0385}	S^{S0385}	S^{S0385}	
hypothetical	1791	174 bp Δ	wt	1 aa change	S^{Fin47_H17}	wt	Variable repeat length

				(aa39)			
map	1981			S^{S0385}	S^{S0385}	S^{S0385}	
sdrH	2069	wt	Δ 39 bp	S^{S0385}	S^{S0385}	S^{S0385}	
hlD	2072	wt	wt	ψ A	wt	wt	Variably present
agrB	2073	wt	wt	A	wt	wt	Variably present
agrA	2076			S^{S0385}	S^{S0385}	S^{S0385}	
fnbB	2550-1	ψ truncated, stop codon	wt	Deleted	Deleted	ψ truncated, stop codon	Often defunct
fnbA	2553	wt	wt	Δ9 bp in repeat region, 12 aa changes	S^{Fin47_H17}	wt	Variable
clfB	2679	ψ truncated	Δ42aa at aa819	wt	wt	wt	Variable repeats / truncated
isaB	2688			S^{S0385}	S^{S0385}	1 SNP,	

						synonymous	
cna	2740	wt	ΔB domain	Δ187aas, 4 X	As Fin47	Δ187aas, 4 X	Variable in this clade